graft-versus-host reactions (GVHD) retaining a graft-versus-leukemia effect and the capacity to reconstitute the immune system. CD6-depleted mbc contain a large proportion of NK and NK-T cells. 63 patients with advanced disease (AML 32, ALL 15, NHL 11, CLL 2, CML 2, SAA 1) were transplanted with marrow from family donors sharing one HLA-haplotype and differing in 0–4 HLA-antigens of the second haplotype. Conditioning consisted of total body irradiation (TBI), antithymocyte globulin (ATG) and cyclophosphamide (CY), post-grafting immunosuppression of cyclosporin A (CSA) and a short course of methotrexate (sMTX). A transfusion of donor leukocytes was given prior to CY. Complete engraftment was observed in 34 evaluable patients given 12 Gy TBI. The dose of TBI could be reduced to 4 Gy without rejection in 25 evaluable patients. GVHD was severe (grade III and IV) in 12 of 48 evaluable patients. An improved method of CD6-depletion was administered to mbc in 9 patients and severe GVHD did not develop. GVHD responded to corticosteroids in most patients. 15 patients survive disease free up to 6 years (median 784 days). Recurrent infections including PTLD were the major cause of transplant-related mortality. Absolute counts of lymphocytes, CD4 and CD8 subpopulations were not different from those of a contemporary group of 46 patients in advanced disease given HLA-identical sibling transplants. However naïve CD4 cells and TRECs were low. Rejection, GVHD and GVL have been controlled by this regimen, but immune reconstitution remains a problem that may be solved by early discontinuation of immunosuppression in this regimen.

GVH/GVL

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ACUTE GRAFT VERSUS HOST DISEASE AFTER NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION (NST) WITH LOW-DOSE TBI, FLUDARABINE AND ANTITHYMOCYTE GLOBULIN (ATG)

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Acute GvHD is a frequent complication of NST although at a lower rate and severity than after full myeloablation. ATG given with conditioning produces substantial host immunosuppression and is thought to produce T-cell depletion of the allograft. We previously showed that the addition of ATG to TBI 200 cGy and fludarabine can improve donor engraftment. Whether it impacts on acute and chronic GvHD and on graft versus tumor effect is less known. Forty-seven pts, not eligible for conventional allogeneic SCT, underwent NST using ATG 15 mg/kg/day (equine) or 1.5 mg/kg/day (rabbit) days 4 to −1, single fraction TBI 200 cGy day −5, fludarabine 30 mg/kg/day days −4 to −2. Oral MMF and CSA started on day −5. Allografts were G-CSF mobilized PBSC. Thirty-two pts (68%) had match related donors, 9 (19%) match unrelated (10/10), 1 (2%) mismatch related (9/10) and 5 (10.6%) mismatch unrelated donors (9/10). Twenty-five pts had sex matched donors (53.2%). Full donor chimerism was documented in 85% and partial (>50%) in 11% of pts. At a median follow up of 309 days, GvHD grade II-IV developed in 13 pts (27.6%). Fatal GvHD of liver and/or gut occurred in 8 pts (17%). The mean age of pts who developed GvHD was 54 years and 57 for those who did not (p = ns). The corresponding mean ages for their donors were 47 vs. 50 years (p = ns). Seven out of fourteen (50%) unrelated transplants recipients and 6/33 (18%) with related donors developed GvHD (p = 0.023). Infectious complications were seen in 8 pts (17%) and were the cause of death in 6 (12%). Thirteen of the 47 pts (27.6%) underwent transplant for AML. Six pts had relapsed/refractory disease at the time of transplant. 3 were in CR2 and 4 in CR1. Eleven pts (84.6%) relapsed with AML after transplant. Previously reported data on NST using 200 cGy of TBI and fludarabine with no ATG showed an incidence of acute GvHD grade II-IV of 64% and 24% GvHD related mortality. Similar rates have been reported with fludarabine and melphalan reduced-intensity conditioning. In our cohort of 47 pts the addition of ATG resulted in decreased incidence of acute GvHD grade II-IV and GvHD related morality without a high rate of infectious complications. A high incidence of disease relapse in pts with AML may reflect the high proportion of pts with advanced leukemia enrolled in the study. However, it also warrants further investigation to rule out that ATG may hinder graft versus leukemia effect.

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SENSITIVE AND QUANTITATIVE CHIMERISM ANALYSIS IN ALL PATIENTS WITH REAL-TIME PCR

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In this study, we wanted to evaluate the predictive value of a sensitive chimerism method for relapse in 45 patients with acute lymphoblastic leukemia (ALL) after allogeneic stem cell transplantation (SCT). The method, based on real-time PCR using single nucleotide polymorphic (SNP) markers, was shown to be more than one log more sensitive than the most common used methods. Mixed chimerism (MC) was detected in the peripheral blood (PB) and bone marrow (BM) samples of all patients that relapsed. However, a high degree of MC was also found in patients without relapse (57% MC in PB and 94% MC in BM). In some patients still in remission, MC was found 4–5 years after SCT. In paired BM-PB samples, the level of recipient cells were more than one log higher in BM as compared to PB. Chimerism results after 3 months post-SCT were associated with relapse. In PB samples, 13/15 patients with a MC level of >0.1% relapsed as compared to 3/22 patients below this level ($p<0.001$). The median time between first detection of this level and relapse was 5.5 (range 0.3–44) months. In BM samples, 10/15 patients with a MC level >1% relapsed as compared to 1/11 patients below this level ($p<0.01$). The median time between first detection of this level and relapse was 18 (1.8–34) months. In conclusion, using a new sensitive chimerism method, a high incidence of MC was found after SCT. Despite this, threshold levels associated with relapse were found both in PB and BM. The time period between first detection of these levels and relapse may be long enough for immunotherapeutic interventions to have an antileukemic effect.

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CLINICAL RELEVANCE OF RECIPIENT LEUKOCYTE INFUSION (RLI) THERAPY

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Background: Surprisingly, anti-tumor responses can occur in patients who reject donor grafts following nonmyeloablative hematopoietic cell transplantation (Dey et al., Biol Blood Marrow Transplant 7:604). In murine mixed chimeras prepared with nonmyeloablative conditioning, we previously showed that recipient leukocyte infusions (RLI) induced anti-tumor responses against host-type tumors (Rubio et al. Blood 102:2300). To further investigate the clinical relevance of this RLI model, we 1. evaluated the effect of RLI from tumor-bearing mice and 2. compared RLI with allogeneic lymphocyte infusion in untreated mice. **Methods:** Mixed chimerism was achieved in BALB/c (H-2^d) mice conditioned with depleting anti-CD4 and CD8 mAbs on Day −5, cyclophosphamide 200 mg/kg on Day −1 and 7 Gy thymic irradiation on Day 0 prior to transplantation of 25×10^6 B10.BR (H-2^k) bone marrow cells. Some groups received RLI (3×10^7 BALB/c spleen cells) seven weeks post-BMT. Some RLI donor mice received BALB/c A20 B cell lymphoma cells (1×10^5) two weeks before RLI. Some groups received RLI depleted of B cells by MACS column for purging tumor cells. A20 cells (5×10^5) were given i.v. one week after RLI in chimeras or after allogeneic lymphocyte infusion (3×10^7 B10.BR spleen cells) to untreated BALB/c mice. **Results:** In the clinical setting, RLI would be obtained from tumor-bearing hosts. We therefore examined whether RLI is still effective when the lymphocytes are obtained from tumor-bearing mice. Recipients of RLI from tumor-bearing mice showed similar tumor survival compared to recipients of RLI